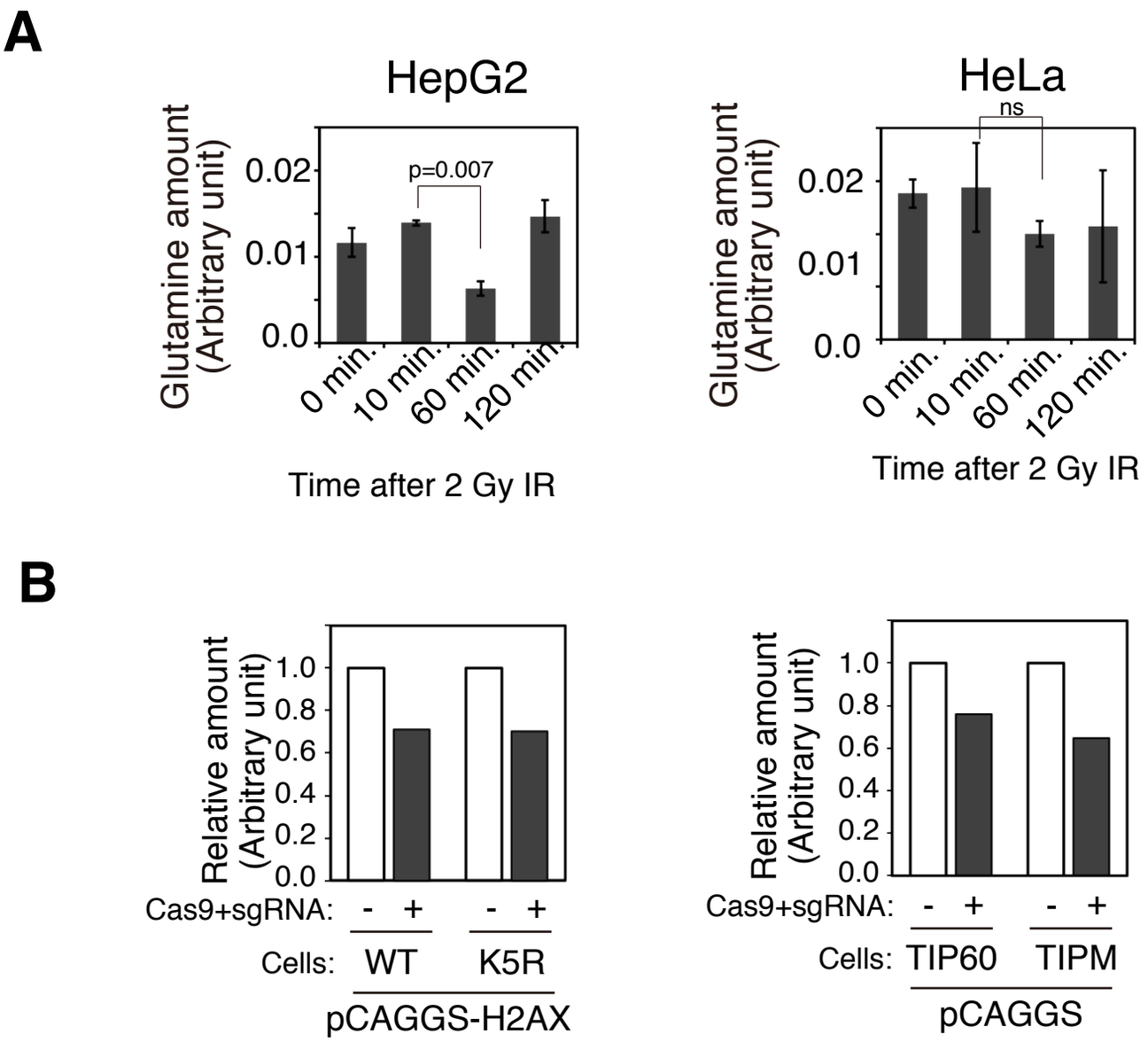

FIG S1 Comparison of glutamine metabolism between HepG2 cells and HeLa cells after IR, and cutting efficiency for ChIP analysis.

(A) Comparison of intracellular levels of glutamine and glutamate in HepG2 and HeLa cells after 2Gy of irradiation. Glutamine consumption was more evident in HepG2 cells compared to HeLa cells, suggesting glutamine dependency of HepG2 cells. For the determination of the concentration, see Methods. Error bars, SD; biological replicates n=3.

(B) Cutting efficiency for ChIP analysis in Figure 1 C, D were shown. qPCR using a primer set across the DSB site was compared to that using a primer to amplify 25 kb away from the DSB site.

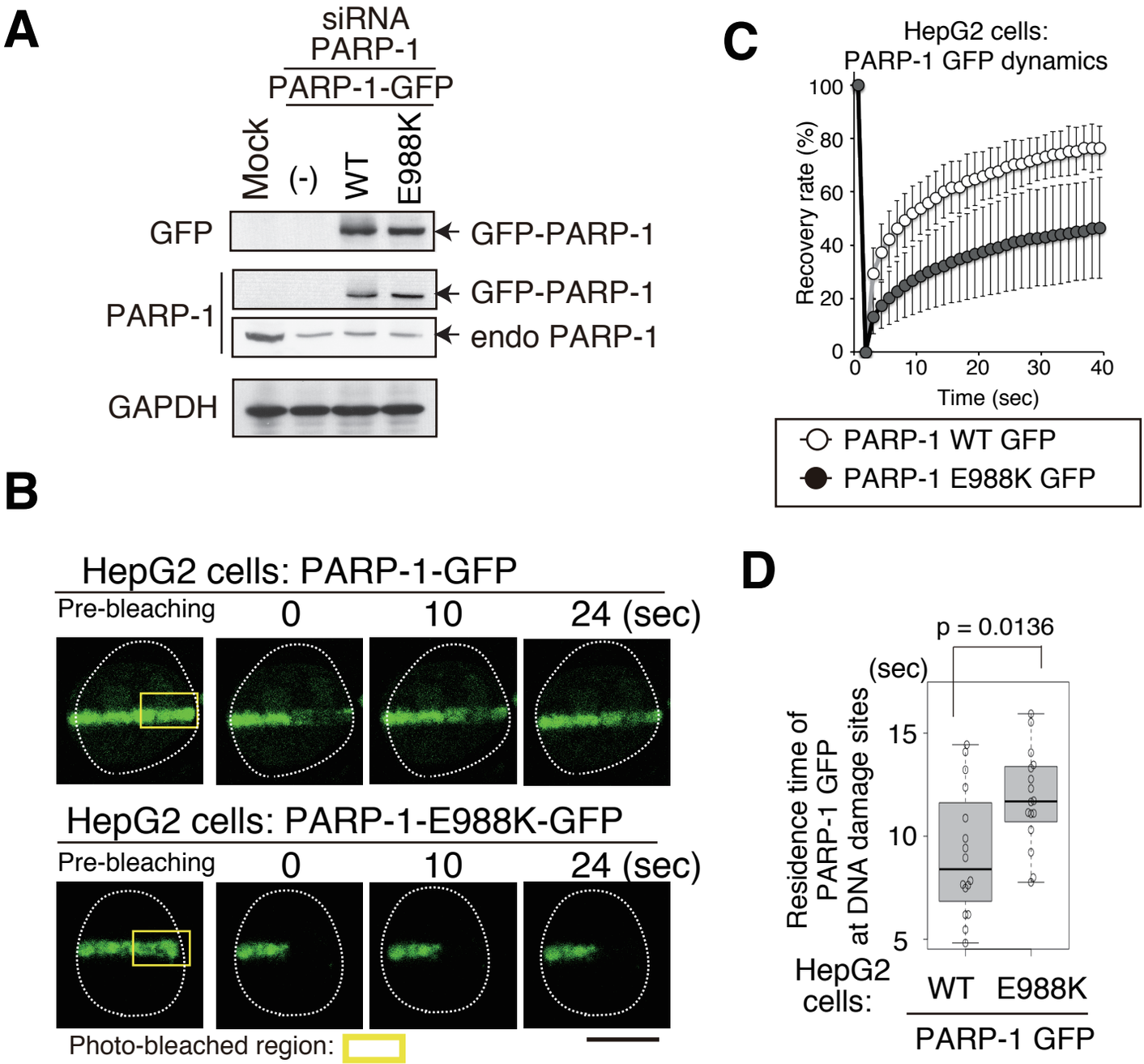

FIG S2 Establishing an experimental system to assess the PARP-1 activity by PARP-1-GFP dynamics in HepG2 cells.

(A) siRNA resistant PARP-1-GFP WT or E988K were stably expressed in HepG2 cells. After knockdown of endogenous PARP-1, Immunoblotting analysis using indicated antibodies were performed. Endo PARP-1; endogenous PARP-1.

(B) FRAP analysis to monitor the dynamics of PARP-1–GFP in HepG2 cells. The PARP-1–GFP WT or that with the E988K mutation accumulated at the microirradiated area was photobleached (yellow box), and the fluorescence recovery was monitored. Bar, 10 μm.

(C) The fluorescence recovery of the GFP signal of PARP-1–GFP WT or E988K was quantified.

(D) The mean residence time of PARP-1–GFP WT or E988K at microirradiated area were calculated and shown in a box plot. p-value, Wilcoxon rank sum test. WT; n=16 , E988K; n=16.

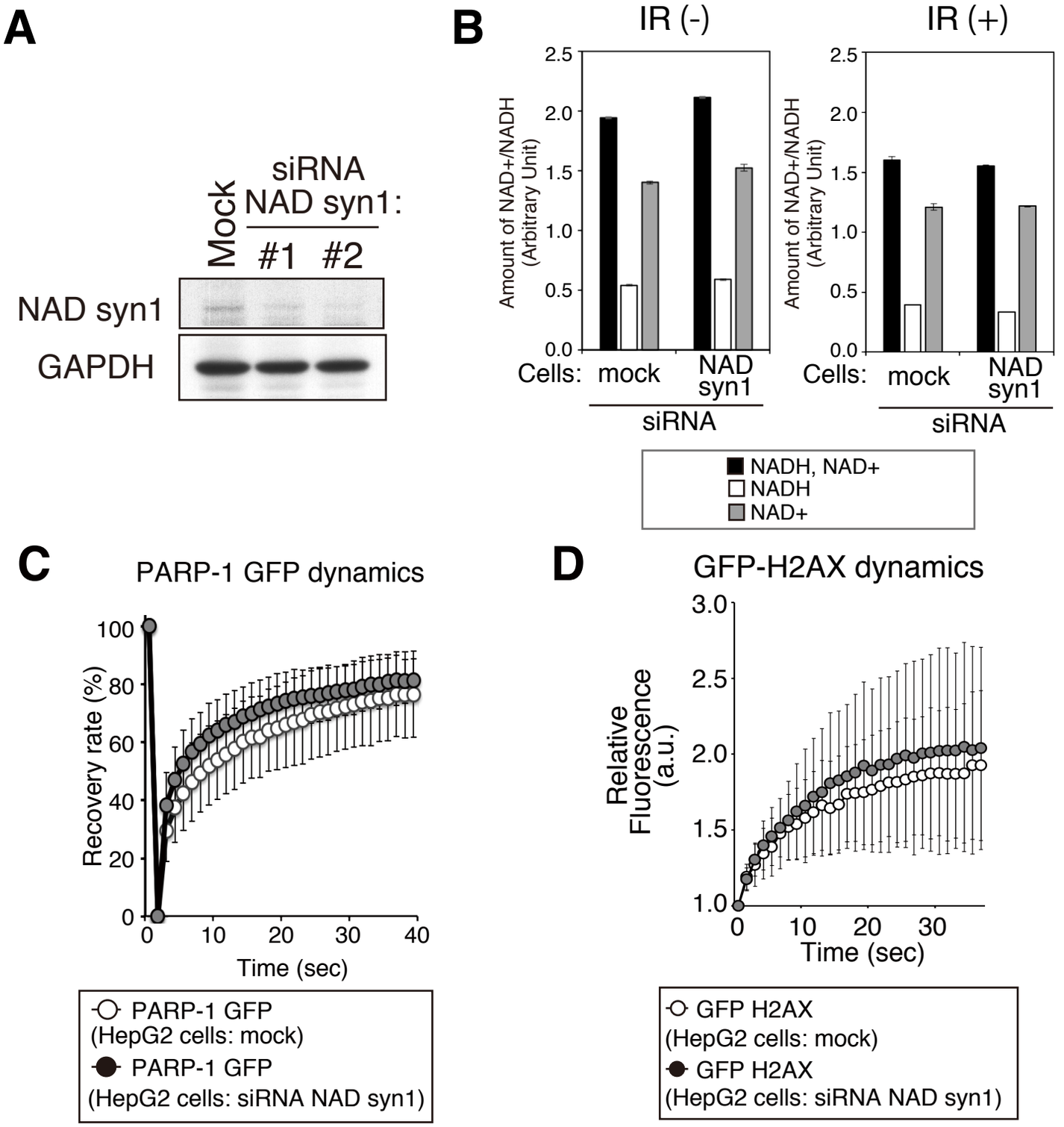

FIG S3 Knock down of NAD syn1 did not alter whole-cell NAD+ content or dynamics of PARP-1 or H2AX at sites of DNA damage.

(A) Confirmation of the knockdown of NAD syn1 by two different siRNAs (#1 and #2). Immunoblots with anti-NAD syn1 and anti-GAPDH are shown. Efficient knock down was seen in #2, thus #2 was used for further analysis.

(B) Intracellular levels of NAD+ and NADH levels were quantified in HepG2 cells transfected with Mock or NAD syn1 siRNA, with (IR (+)) or without (IR (-)) 2Gy-irradiation followed by a 10-min recovery prior to the harvest of cells. For the determination of the concentration, see Materials and Methods. Error bars, SD (n=3).

(C) The fluorescence recovery of the GFP signal of PARP-1–GFP WT in control HepG2 cells (mock) or HepG2 cells transfected with NAD syn1 siRNA was quantified.

(D) The accumulation of GFP-H2AX at microirradiated area was quantified in control HepG2 cells (mock) or HepG2 cells transfected with NAD syn1 siRNA. control HepG2 cells; n=12, NAD syn1 siRNA; n=15)

Ikura M. et al., Figure S3

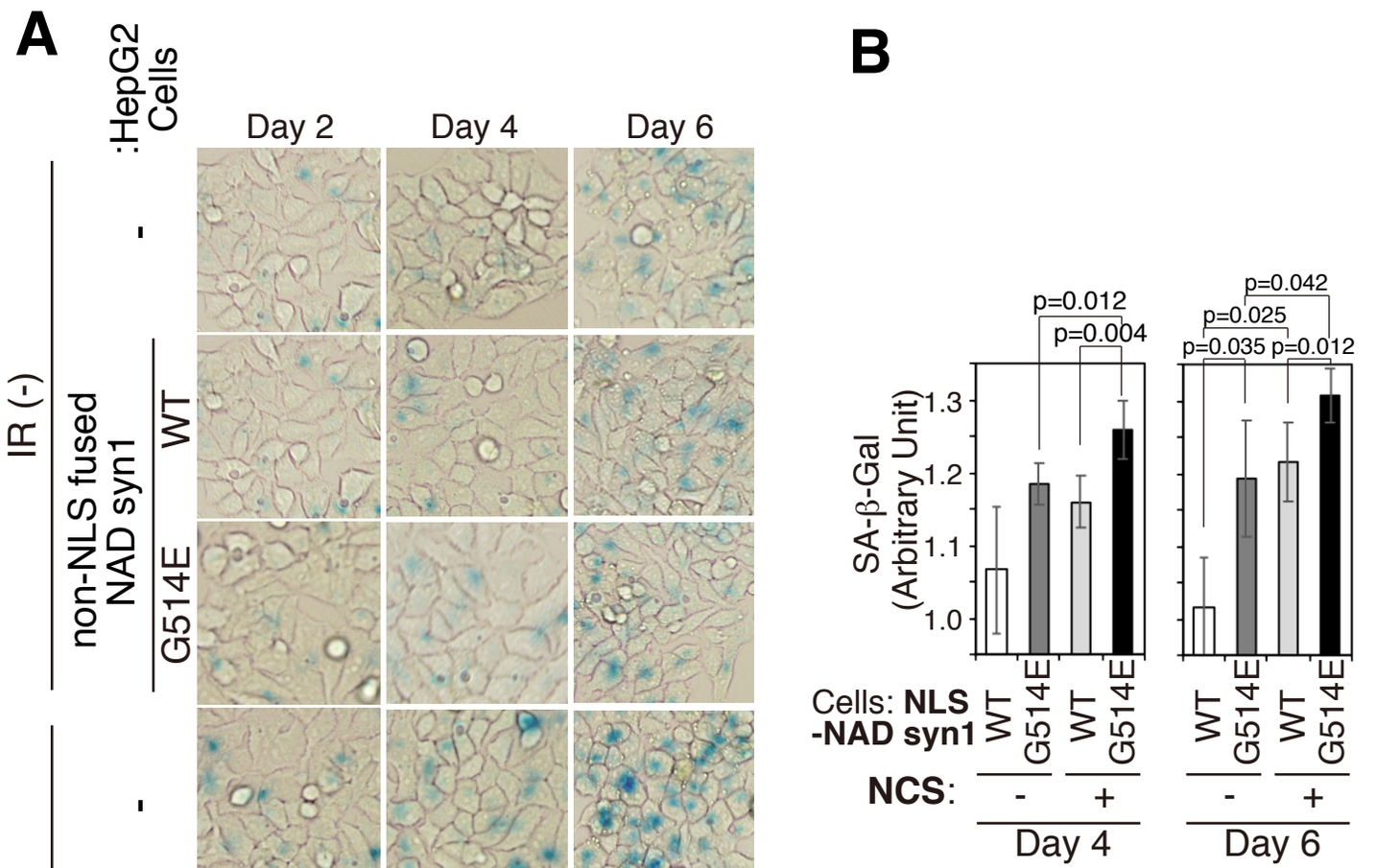

FIG S4 Cellular senescence assay in non-NLS fused NAD syn1-WT or -G514E expressing cells after IR, and NLS fused NAD syn1-WT or -G514E expressing cells after neocarzinostatin (NCS)
(A) HepG2 cells expressing non-NLS fused NAD syn1-WT or -G514E (NAD syn1-WT or -G514E) or just HepG2 cells (-) were cultured after the 2 Gy-irradiation (IR), and stained for SA-β-gal. Days 2, 4, 6 correspond to 2, 4, 6 days after the IR. Bar: 250 μm.
(B) SA-β-gal activity in the HepG2 cells expressing NLS-NAD syn1-WT or -G514E after neocarzinostatin (NCS) treatment were quantified using a SPiDER-b-gal kit (DOJINDO). The SA-β-gal activity is indicated as the ratio to the no cell control background. Error bars, SD, p-values; student T-test, n=4, technical replicates. See Methods for details.

Ikura M. et al., Figure S4

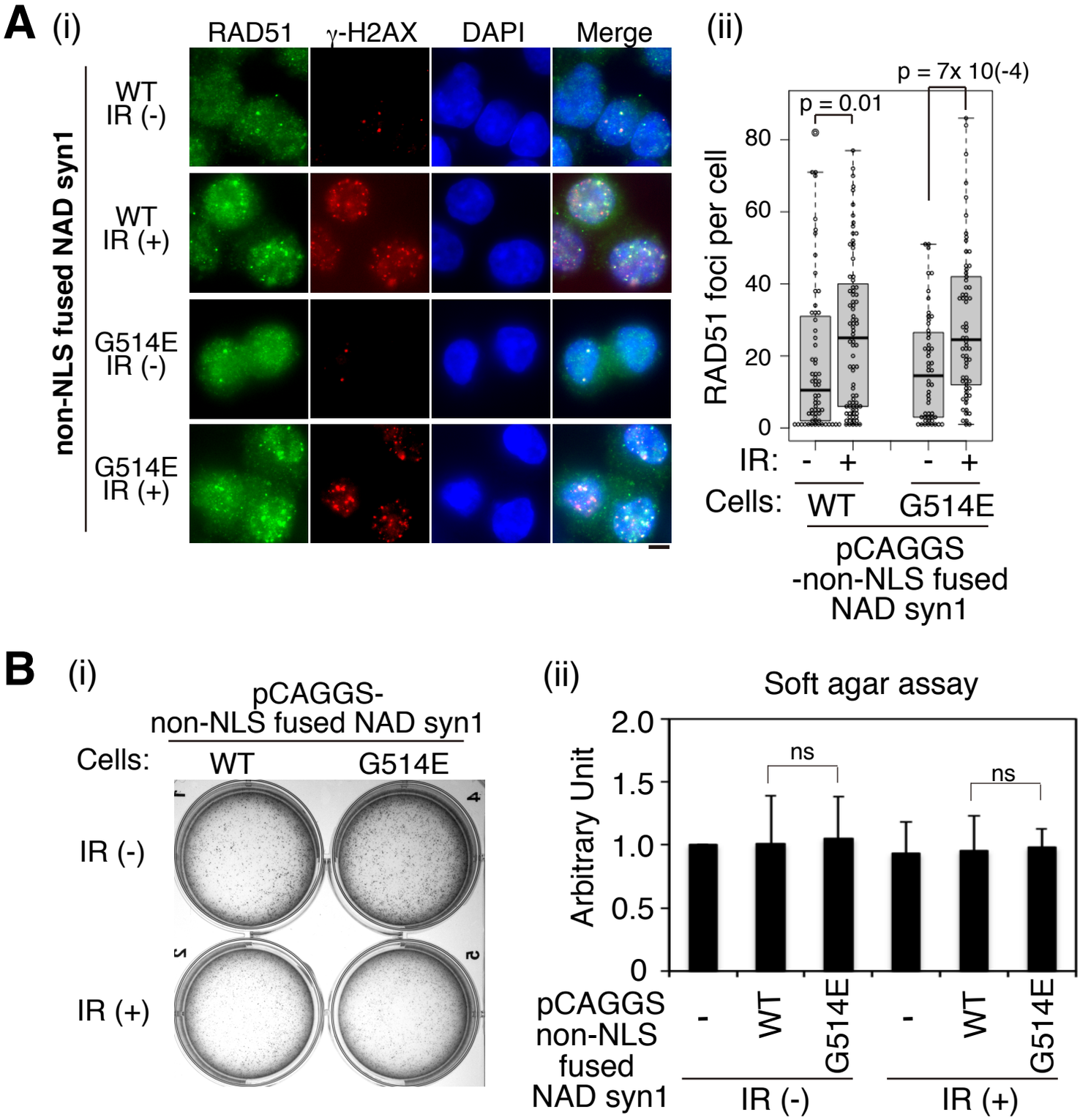

FIG S5 RAD51 foci and soft agar assay in non-NLS fused NAD syn1-WT or -G514E expressing cells after IR

(A) (i) Immunohistochemistry analysis using an antibody against RAD51, in cells expressing non-NLS fused NAD syn1-WT or -G514E (NAD syn1-WT or -G514E) with (IR (+)) without (IR (-)) 3Gy-irradiation followed by a 60-min recovery. Cell images are shown. (ii) The quantification of the number of RAD51 foci per cells were shown. WT: IR(-); n=138, IR (+); n=131 G514E: IR(-); n=127, IR (+); n=113. Cells which do not have RAD51 foci were omitted from the graphs. See materials and methods for details. P-values, Wilcoxon rank sum test.

(B) (i) A soft agar growth assay in the non-NLS fused NAD syn1-WT (WT) or -G514E (G514E) expressing HepG2 cells with or without 3Gy IR prior to the assay. (ii) Quantification of cell growth in a soft agar growth assay in (i). n=3, error bar, SD. P-values; Students' T-test.

Ikura M. et al., Figure S5